Citation:

Thijssen MA, Mensink RP. Small differences in the effects of stearic acid, oleic acid, and linoleic acid on the serum lipoprotein profile of humans. *Am J Clin Nutr.* 2005 Sep; 82(3): 510-516.

PubMed ID: 16155261

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To compare simultaneously the effects of stearic, oleic and linoleic acids on the serum lipoprotein profile of healthy subjects.

Inclusion Criteria:

- Healthy (based on medical questionnaire)
- 18 to 69 years
- Non-smokers
- Weight stable
- Body mass index (BMI) less than 32kg/m^2
- Systolic blood pressure (SBP) less than 160mmHg
- Diastolic blood pressure (DBP) less than 95mmHg
- Total cholesterol: 5.0 to 8.0mmol per L
- Serum triacylglycerol less than 4.0mmol per L.

Exclusion Criteria:

Subjects with a history of:

- Atherosclerotic disease
- Glycosuria, proteinuria
- Anemia
- Taking medications known to affect blood lipids or hemostatic variables.

Description of Study Protocol:

Recruitment

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

Subjects were recruited via advertisements in local newspapers, in a university hospital newsletter and via posters in university buildings.

Design

Randomized, multiple, crossover (five weeks for each diet).

Dietary Intake/Dietary Assessment Methodology

- High stearic acid diet (18:0): 60% from experimental products (9% of palm oil, 5.5% safflower oil, 5.0% olive oil, 33.5% cocoa butter, 18.0% high-oleic acid sunflower oil and 29.0% glycerol tristearate)
- High oleic acid diet (18:1): 60% from experimental products (19.5% of palm oil, 26.0% olive oil, 7.5% cocoa butter, 4.7% high-oleic acid sunflower oil and 47.0% glycerol tristearate)
- High linoleic acid diet (18:2): 60% from experimental products (20.0% of palm oil, 52.0% safflower oil, 7.0% olive oil, 9.0% cocoa butter, 12.0% high-oleic acid sunflower oil and 29.0% glycerol tristearate)
- All three diets were same for the nutrient composition and had a 7% difference in total energy provided by stearic acid, oleic acid or linoleic acid
- Total fat intake was targeted at 37% of total energy intake for all three diets
- 60% of total fat energy was from experimental fats (produced by NIZO Food Research, Ede, Netherlands) and 40% of total fat energy was from "free-choice" fat-containing products.

Intervention

- Three diet treatments
- Six diet groups
- Randomized (blocked by sex)
- Five weeks for each diet
- Total energy intake was estimated with the Harris-Benedict equation
- More than one week for washout period between diet treatment
- Subjects were asked not to change their physical activity
- Subjects were asked to visit the study center every week to receive a new supply and to be weighed
- Subjects were asked to keep food diary and to complete a food-frequency questionnaire (FFQ) every week.

Statistical Analysis

- Main analysis was performed using general linear model procedure of SPSS 11
- $P<0.05$ was considered as significant
- Differences in effects on lipid and lipoprotein were examined with diet and period as fixed factor and subject number as a random factor
- Interaction terms of diet and sex or diet and BMI were created to test the effect modification.

Data Collection Summary:

Timing of Measurements

Two serum samples at week four and week five were averaged.

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

Dependent Variables

- Total cholesterol (mmol per L): Measured the serum concentration (ABX Diagnostics, Montpellier)
- LDL-cholesterol (mmol per L): Measured the serum concentration (precipitation method, Roche Diagnostics Corporation)
- HDL-cholesterol (mmol per L): Measured the serum concentration (precipitation method, Roche Diagnostics Corporation)
- Triacylglycerols (mmol per L): Measured the serum concentration (Sigma Aldrich Chemie)
- Apolipoprotein A-I (g per L): Measured the serum concentration by using a immunoturbidimetric method ABX Diagnostics, Montpellier)
- Apolipoprotein B (g per L): Measured the serum concentration by using a immunoturbidimetric method ABX Diagnostics, Montpellier)
- Total:HDL cholesterol:
- Lipoproyrin particles: Analyzed in a randomly chosen subset of 22 subjects (stratified for sex, nine men and 13 women) by NMR spectroscopy.

Independent Variables

Three diet treatments.

Control Variables

- Sex
- BMI.

Description of Actual Data Sample:

- *Initial N:* 58 eligible [withdrawal reasons related to the strict study protocol (N=4); stressful personal or job circumstance (N=5); physical illness (N=3); did not follow the protocol (N=1)]
- *Attrition (final N):* 45 (18 men, 27 women)
- *Age:* 28 to 66 years
- *Other relevant demographics:* Six women were post-menopausal and five used oral contraceptive
- *Anthropometrics:* BMI range from 18 to 29.8kg/m^2
- *Location:* Maastricht University, Maastricht, Netherlands.

Summary of Results:

- Mean daily energy intake and the composition of the three diets, as determined by the FFQs agreed well with the prescribed composition of the diets and intakes of test products (bread, cake and margarines) did not differ between diets
- Total fat intake, on average, was 38% of energy and did not differ between the three diets (P=0.701). The nutrient composition of the diets also did not differ, except that 7% of energy was provided by different fats (stearic, oleic, or linoleic acid)
- Mean body weights at the end of each dietary period did not differ significantly between the three diets (P=0.449) and were 72.5±13.0kg with the stearic acid diet, 72.5±13.2kg with the oleic acid diet and 72.7±12.9kg with the linoleic acid diet
- Effects on HDL-cholesterol (P=0.866) and triacylglycerol (P=0.670) concentrations did not

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

differ between the three diets
- No significant (NS) differences existed between the three diets (P=0.303) with respect to the total to HDL-cholesterol ratio
- Changes in concentrations of apo B (P=0.122) and A-I (P=0.534) were also not statistically significant between the three diets, paralleling those of LDL and HDL-cholesterol, respectively
- A statistically significant diet-x-BMI interaction effect (P=0.029) was observed for apo B. In the high BMI group (P=0.011 for diet effects), the linoleic acid diet reduced apo B concentrations by 0.08g per L relative to stearic acid (P=0.010; 95% CI for the difference: 0.02, 0.15 g per L). In the low BMI group, apo B concentrations did not differ between the three diets (P=0.689). None of the dietary effects differed significantly between men and women (data not shown).

Serum Lipid and Lipoprotein Concentrations and the Ratio of Total to HDL-cholesterol During Consumption of Diets Enriched in Stearic, Oleic and Linoleic Acids for Five Weeks

Variables	Stearic Acid Diet	Oleic Acid Diet	Linoleic Acid Diet	P for the Diet Effect
	Mean (SD)	Mean (SD)	Mean (SD)	
Total cholesterol (mmol per L)	5.81 (0.94)	5.73 (0.81)	5.66 (0.91)	0.110
LDL-cholesterol (mmol per L)	3.79 (0.91)	3.71 (0.79)	3.65 (0.91)	0.137
HDL-cholesterol (mmol per L)	1.45 (0.43)	1.46 (0.45)	1.46 (0.44)	0.866
Triacyglycerols (mmol per L)	1.24 (0.55)	1.22 (0.52)	1.21 (0.60)	0.670
Apolipoprotein A-I (g per L)	1.39 (0.23)	1.41 (0.25)	1.40 (0.244)	0.534
Total:HDL	4.31 (1.33)	4.22 (1.23)	4.19 (1.28)	0.303

Author Conclusion:

With realistic intakes of stearic, oleic and linoleic acids, differences between their effects on the serum lipoprotein profile are small.

Reviewer Comments:

- *Overall, the study was well-done*
- *One major concern is their assignment of the experimental diets. The authors mentioned this is a multiple randomized cross-over study. There are three experimental diets but six treatment when subjects were randomized. It is not clear in the text how this was done*
- *It seems like each diet has two test periods (five weeks for each), but how this was treated in*

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

the analysis is not clear.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	???
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	N/A
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	???
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	???
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	N/A
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	N/A
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	No
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12

8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	No
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	No
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	No

© 2012 USDA Evidence Analysis Library. Printed on: 09/24/12